

APPLICATION FOR UNITED STATES LETTERS PATENT
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

(Case No. 99-898-A)

Title: Oxo-imidazopyridine-carboxamides

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Oxo-imidazopyridine-carboxamides

Background of the Invention

5 This application claims priority from U.S. Provisional Application S.N. 60/209,855, filed May 26, 2000, the disclosure of which is incorporated herein in its entirety.

Field of the Invention

10 This invention relates to oxo-imidazopyridine-carboxamides that bind with high selectively and high affinity to the benzodiazepine site of GABA_A receptors. This invention also relates to pharmaceutical compositions comprising such compounds and to the use of such compounds in treatment of 15 central nervous system (CNS) diseases.

Description of the Related Art

The GABA_A receptor super-family represents one of the classes of receptors through which the major inhibitory 20 neurotransmitter, γ -aminobutyric acid, or GABA, acts. Widely, although unequally, distributed through the mammalian brain, GABA mediates many of its actions through a complex of proteins called the GABA_A receptor, which causes alteration in chloride conductance and membrane polarization. In addition to 25 being the site of neurotransmitter action, a number of drugs including the anxiolytic and sedating benzodiazepines bind to this receptor. The GABA_A receptor comprises a chloride channel that generally, but not invariably, opens in response to GABA, allowing chloride to enter the cell. This, in turn, effects a 30 slowing of neuronal activity through hyper-polarization of the cell membrane potential.

GABA_A receptors are composed of five protein subunits. A number of cDNAs for these GABA_A receptor subunits have been cloned and their primary structures determined. While these

subunits share a basic motif of 4 membrane-spanning helices, there is sufficient sequence diversity to classify them into several groups. To date at least 6 α , 3 β , 3 γ , 1 ϵ , 1 δ and 2 ρ subunits have been identified. Native GABA_A receptors are 5 typically composed of 2 α , 2 β , and 1 γ subunits (Pritchett & Seuberg *Science* 1989; 245:1389-1392, and Knight et. al., *Recept. Channels* 1998; 6:1-18). Various lines of evidence (such as message distribution, genome localization and biochemical study results) suggest that the major naturally 10 occurring receptor combinations are $\alpha_1\beta_2\gamma_2$, $\alpha_2\beta_3\gamma_2$, $\alpha_3\beta_3\gamma_2$, and $\alpha_5\beta_3\gamma_2$ (Mohler et al. *Neuroch. Res.* 1995; 20(5):631-36).

The GABA_A receptor binding sites for GABA (2 per receptor complex) are formed by amino acids from the α and β subunits. Amino acids from the α and γ subunits together form 15 1 benzodiazepine site per receptor. Benzodiazepines exert their pharmacological actions by interacting with the benzodiazepine binding sites associated with the GABA_A receptor. In addition to the benzodiazepine site (sometimes referred to as the benzodiazepine or BDZ receptor), the GABA_A receptor contains 20 sites of interaction for several other classes of drugs. These include a steroid binding site, a picrotoxin site, and a barbiturate site. The benzodiazepine site of the GABA_A receptor is a distinct site on the receptor complex that does not overlap with the site of interaction for other classes of 25 drugs that bind to the receptor or for GABA (see, e.g., Cooper, et al., *The Biochemical Basis of Neuropharmacology*, 6th ed., 1991, pp. 145-148, Oxford University Press, New York).

In a classic allosteric mechanism, the binding of a drug to the benzodiazepine site increases the affinity of the GABA 30 receptor for GABA. Benzodiazepines and related drugs that enhance the ability of GABA to open GABA_A receptor channels are known as agonists or partial agonists depending on the level of GABA enhancement. Other classes of drugs, such as β -

carboline derivatives, that occupy the same site and negatively modulate the action of GABA are called inverse agonists. A third class of compounds exists. These compounds occupy the same site as both the agonists and inverse agonists 5 and yet have little or no effect on GABA activity. These compounds will, however, block the action of agonists or inverse agonists and are thus referred to as GABA_A receptor antagonists.

The important allosteric modulatory effects of drugs 10 acting at the benzodiazepine site were recognized early, and the distribution of activities at different subtype receptors has been an area of intense pharmacological discovery. Agonists that act at the benzodiazepine site are known to exhibit anxiolytic, sedative, and hypnotic effects, while 15 compounds that act as inverse agonists at this site elicit anxiogenic, cognition enhancing, and proconvulsant effects. While benzodiazepines have enjoyed long pharmaceutical use as anxiolytics, these compounds are known to exhibit a number of unwanted side effects. These include cognitive impairment, 20 sedation, ataxia, potentiation of ethanol effects, and a tendency for tolerance and drug dependence.

GABA_A selective ligands also act to potentiate the effects of certain other CNS active compounds. For example, there is evidence that selective serotonin reuptake inhibitors (SSRIs) 25 display greater antidepressant activity when used in combination with GABA_A selective ligands than when used alone.

SUMMARY OF THE INVENTION

This invention provides oxo-imidazopyridine-carboxamide derivatives that bind with high affinity and high selectivity 5 to the benzodiazepine site of GABA_A receptors, including human GABA_A receptors. Compounds of the invention bind, preferably with high selectivity and affinity, to GABA_A receptors and thereby act as agonists, antagonists or inverse agonists of such receptors. As such, they are useful in the treatment of 10 various CNS disorders.

The compounds of the invention bind with high selectivity and high affinity to the benzodiazepine site of GABA_A receptors.

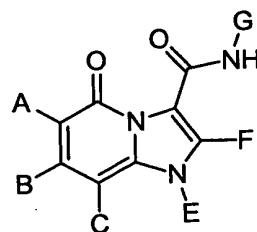
The invention provides compounds of Formula I (shown 15 below), and pharmaceutical compositions comprising compounds of Formula I.

The invention further comprises methods of treating patients suffering from certain CNS disorders with an effective amount of a compound of the invention. The patient 20 may be a human or other mammal. Treatment of humans, domesticated companion animals (pets) or livestock animals suffering from certain CNS disorders with an effective amount of a compound of the invention is encompassed by the invention.

25 In a separate aspect, the invention provides a method of potentiating the actions of other CNS active compounds. This method comprises administering an effective amount of a compound of the invention in conjunction with the administration of another CNS active compound.

30 In another, aspect this invention relates to the use of compounds of Formula I as probes for the localization of GABA_A receptors in tissue sections. Such probes may be used *in vitro* (e.g., in binding assays) or *in vivo* (e.g., in PET or SPECT Scans).

Accordingly, in a broad aspect, the invention is directed to compounds of Formula I



I

5 and the pharmaceutically acceptable non-toxic salts thereof wherein:

A, B and C independently represent hydrogen, halogen, C₁-C₆ alkyl,

10 wherein said C₁-C₆ alkyl is straight, branched or cyclic, contains zero, one or two double or triple bonds, and is unsubstituted or substituted with one or more substituents selected from hydroxy, oxo, halogen, amino, mono- or di(C₁-C₆)alkylamino, C₁-C₃ alkoxy, and C₃-C₇ cycloalkyl,

15 C₁-C₆ alkoxy, C₁-C₆ haloalkyl, hydroxy, amino, mono- or di(C₁-C₆)alkylamino, C₃-C₇ cycloalkyl, mono- or di(C₁-C₆)alkylamino(C₁-C₆)alkyl, mono- or di(C₁-C₆)alkylamino(C₁-C₆)alkoxy, aryl(C₁-C₆)alkyl and substituted aryl(C₁-C₆)alkyl;

E is selected from hydrogen, hydroxy, C₁-C₆alkyl, and C₁-C₆

20 C₆ mono- or di(C₁-C₆)alkyl amino(C₁-C₆)alkyl;

F is selected from hydrogen, halogen, hydroxy, amino, and C₁-C₆ alkyl;

25 G is selected from aryl and heteroaryl, each of which is optionally substituted with up to three groups independently selected from the group consisting of halogen, C₁-C₆ alkyl, C₁-C₆ alkoxy, C₁-C₆ haloalkyl, hydroxy, mono- or di(C₁-C₆)alkylamino, and C₁-C₆ alkyl substituted with one or two groups independently selected from -OR², -NR⁶R⁷, and heterocycloalkyl, where R², R⁶ and R⁷ are the same or different

and represent hydrogen, C₁-C₆ alkyl, or C₃-C₇ cycloalkyl, or NR⁶R⁷ represents a cyclic moiety having 3-7 members.

In another aspect, the invention provides intermediates useful for preparing the compounds of Formula I.

In a further aspect, the invention provides methods for making compounds of Formula I.

Detailed Description of the Invention

The compounds encompassed by the invention are represented by general Formula I (set forth above). The invention includes the pharmaceutically acceptable salts of 5 those compounds.

Preferred compounds of Formula I (hereinafter compounds of Formula IA) are those where

A, B and C are independently chosen from

(i) hydrogen, halogen, C₁-C₆ alkoxy, C₁-C₆ haloalkyl, 10 hydroxy;

(ii) C₁-C₆ alkyl, C₃-C₇ cycloalkyl, C₂-C₆ alkenyl, or C₂-C₆ alkynyl,

where each alkyl, cycloalkyl, alkenyl, or alkynyl is 15 optionally substituted with one or more of hydroxy, oxo, halogen, amino, C₁-C₆ haloalkyl, C₃-C₇ cycloalkyl, C₁₋₃ alkoxy, or mono- or dialkylamino;

(iii) R³R⁴N- where

R³ and R⁴ independently represent hydrogen, C₁-C₆ alkyl, 20 amino(C₁-C₆)alkyl, (C₁-C₆)alkoxy(C₁-C₆)alkyl, or C₃-C₇ cycloalkyl; or

NR³R⁴ represents heteroaryl or heterocycloalkyl;

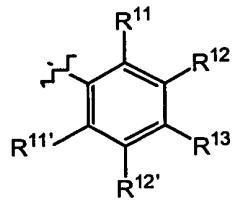
E is hydrogen; or

E is C₁-C₆ alkyl, amino(C₁-C₆)alkyl, mono or di(C₁-C₆)alkyl, 25 amino(C₁-C₆)alkyl, or C₁-C₆alkoxy(C₁-C₆)alkyl, each of which alkyl portion being unsubstituted or substituted with one or more of halogen, hydroxy, C₃-C₇ cycloalkyl, aryl, heterocycloalkyl, or heteroaryl;

F is selected from hydrogen, halogen, hydroxy, amino, and C₁-C₆ 30 alkyl; and

G is selected from:

(i) a group of the formula



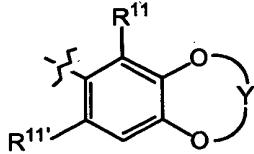
where R^{11} , $R^{11''}$, R^{12} , $R^{12''}$, and R^{13} are the same or different and are selected from

5 hydrogen, halogen, C₁-C₆ alkyl, hydroxy, trifluoromethyl,
-OR², and -NR⁶R⁷

where R^2 , R^6 and R^7 are the same or different and are selected from hydrogen, C_1-C_6 alkyl, and C_3-C_7 cycloalkyl; or

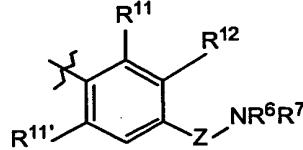
NR⁶R⁷ represents aryl or heterocycloalkyl;

10 (ii) a group of the formula:



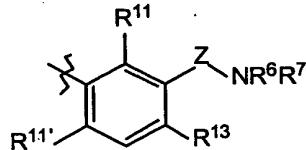
where Y is C_1-C_6 alkylene, and R^{11} and $R^{11'}$ are as defined above;

15 (iii) a group of the formula:



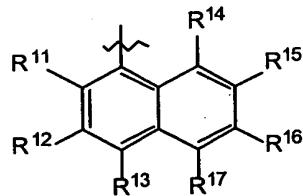
where R^6 , R^7 , R^{11} , and $R^{11'}$ are as defined above; and Z is C_1-C_6 alkylene or C_1-C_6 alkyleneoxy;

20 (iv) a group of the formula:



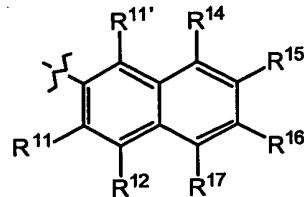
where Z , R^6 , R^7 , R^{11} , $R^{11'}$, and R^{13} are as defined above;

(v) a group of the formula:



5 where R^{11} , R^{12} , and R^{13} are as defined above, and R^{14} , R^{15} , R^{16} , and R^{17} are the same or different and independently carry the same definition as R^{11} ;

(vi) a group of the formula:



10 where R^{11} , $R^{11'}$, R^{12} , R^{14} , R^{15} , R^{16} , and R^{17} are as defined above;

(vii) a group of the formula:

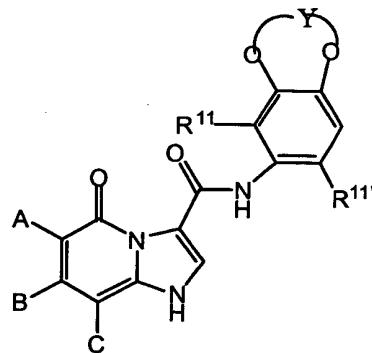


where Q represents a heteroaryl group.

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In specific embodiments, Z is methylene, ethylene, methyleneoxy or ethyleneoxy.

The invention encompasses compounds of Formula III:

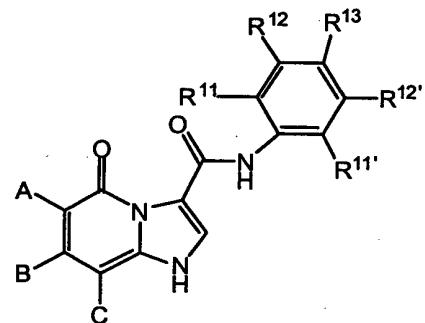


III

where A, B, C, R¹¹, R^{11'}, and Y are as defined above for Formula IA.

5

Thus, the invention encompasses compounds of Formula II:

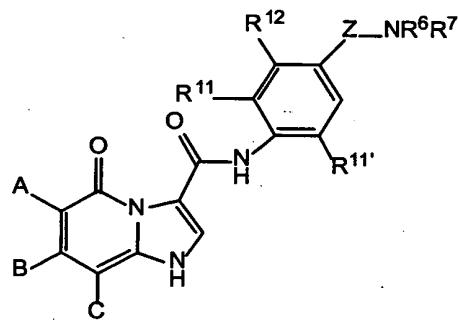


II

where A, B, C, R¹¹, R^{11'}, R¹², R^{12'} and R¹³ are as defined above for Formula IA.

10

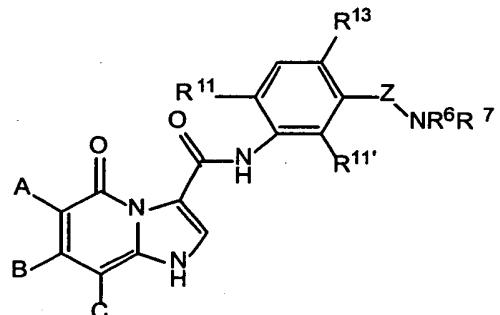
The invention encompasses compounds of Formula IV:



IV

where A, B, C, R¹¹, R^{11'}, R¹², Z, R⁶, and R⁷ are as defined above for Formula IA.

The invention encompasses compounds of Formula V:



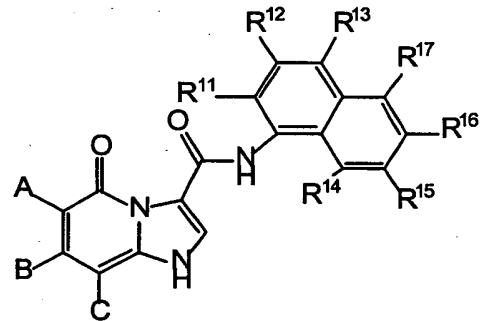
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V

where A, B, C, R¹¹, R^{11'}, R¹³, Z, R⁶, and R⁷ are as defined above for Formula IA.

10

The invention encompasses compounds of Formula VI:

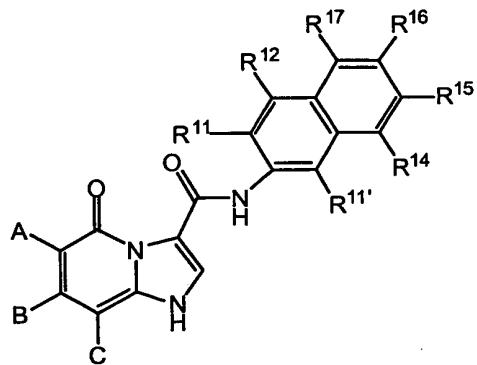


VI

where A, B, C, R¹¹, R¹², R¹³, R¹⁴, R¹⁵, R¹⁶, and R¹⁷ are as defined above for Formula IA.

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The invention encompasses compounds of Formula VII:

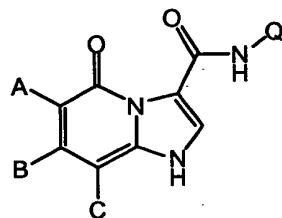


VII

where A, B, C, R¹¹, R¹², R^{11'}, R¹⁴, R¹⁵, R¹⁶, and R¹⁷ are as defined above for Formula IA.

5

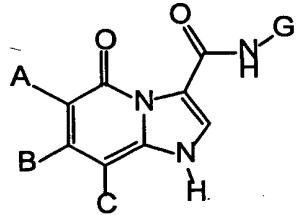
The invention encompasses compounds of Formula VIII:



VIII

where A, B and C are as defined above for Formula IA; and
10. Q is heteroaryl.

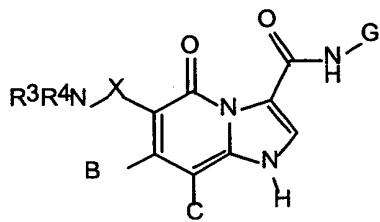
The invention encompasses compounds of Formula IX:



IX

15 where A, B, C and G are as defined above for Formula IA.

The invention encompasses compounds of Formula X:

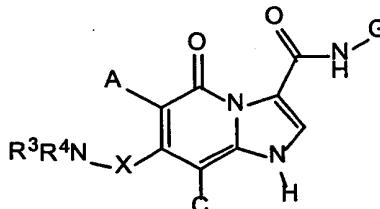


X

where R^3 , R^4 , B, C, and G are as defined above for Formula IA, and X is C_1-C_6 alkylene.

5

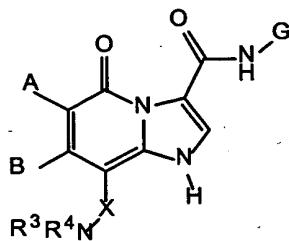
The invention encompasses compounds of Formula XI:



XI

where A, C, G, R^3 , and R^4 are as defined above for Formula IA, 10 and X is C_1-C_6 alkylene.

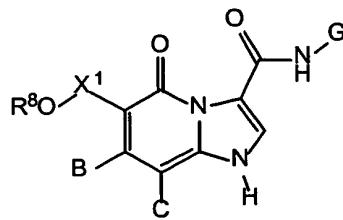
The invention encompasses compounds of Formula XII:



XII

15 where A, B, G, R^3 , and R^4 are as defined above for Formula IA, and X is C_1-C_6 alkylene.

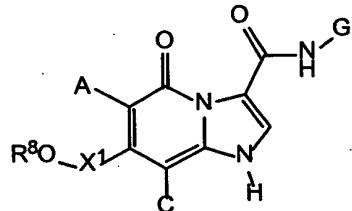
The invention encompasses compounds of Formula XIII:



XIII

where B, C, and G are as defined above for Formula IA, R^8 is defined the same as R^2 , and X^1 is C_1-C_6 alkylene or C_1-C_6 alkyleneamino.

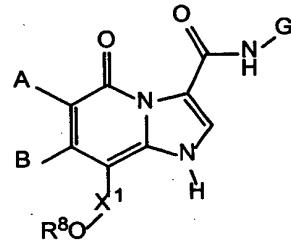
The invention encompasses compounds of Formula XIV:



XIV

where A, C, and G are as defined above for Formula IA, R^8 is defined the same as R^2 , and X^1 is C_1-C_6 alkylene or C_1-C_6 alkyleneamino .

The invention encompasses compounds of Formula XV:



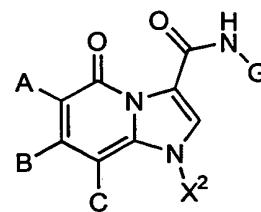
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XV

where A, B, and G are as defined above for Formula IA, R^8 is defined the same as R^2 , and X^1 is C_1-C_6 alkylene or C_1-C_6 alkylene amino.

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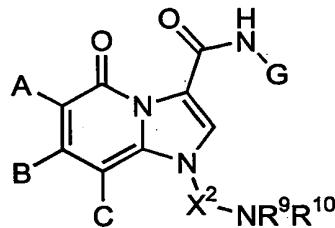
The invention encompasses compounds of formula XVI:



XVI

where A, B, C, and G, X² is C₁-C₆alkyl.

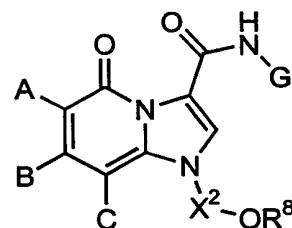
5 The invention encompasses compounds of Formula XVII:



XVII

where A, B, C, and G are as defined above for Formula IA, and R⁹ and R¹⁰ are defined the same as R³ and R⁴, and X² is C₁-C₆alkylene.

The invention encompasses compounds of Formula XVIII:

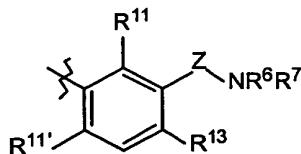


XVIII

15 where A, B, C, and G are as defined above for Formula IA, and R⁸ is defined the same as R², and X² is C₁-C₆alkylene.

Specific X groups are methylene and ethylene groups.
 Specific X¹ groups are methylene, ethylene, methyleneamino and
 20 ethyleneamino groups.

Preferred compounds of general Formula I are those where G is the group:



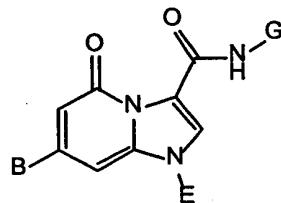
Z is alkyleneoxy or alkylene; and

5 NR⁶R⁷ represents a 5- or 6- membered heteroaryl ring.

Such preferred compounds are hereinafter referred to as compounds of Formula IB. Particularly preferred compounds of Formula IB are those where the 5- or 6- membered heteroaryl ring is imidazole, triazole, or pyrazole.

10

Other preferred compounds of Formula I are those of the formula:



where

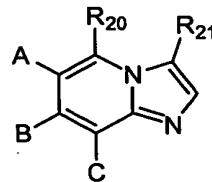
15 E and G are as defined above for Formula IA or for Formula IB; and

B is hydrogen or lower alkyl.

In this latter group of compounds, B is preferably hydrogen or C₁-C₂ alkyl, and most preferably hydrogen or methyl.

20

The invention further encompasses compounds of formula XIX



Formula XIX

where

A, B and C are as defined for Formula I or IB;

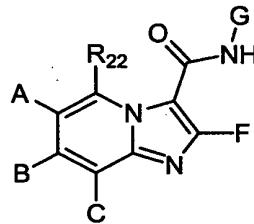
R₂₀ represents halogen, C₁-C₆ alkoxy, or benzyloxy; and

R₂₁ represents hydrogen or halogen.

5

Particular compounds of Formula XIX are those where A and C are hydrogen, R₂₀ is halogen, preferably bromo, and R₂₁ is hydrogen. Other particular compounds of Formula XIX, are those where R₂₀ is C₁-C₆ alkoxy, preferably methoxy, and R₂₁ is hydrogen, or halogen. Still other particular compounds of Formula XIX, are those where A and C are hydrogen, R₂₀ is C₁-C₆ alkoxy, preferably methoxy, R₂₁ is halogen, preferably bromo, and B is C₁-C₆ alkyl.

15 The invention also encompasses compounds of Formula XX,



Formula XX

where

A, B, C, E, G and F carry the same definitions as for Formula

20 I or IB; and R₂₂ represents benzyloxy or C₁-C₆ alkoxy, preferably methoxy.

Preferred compounds of Formula XX include those where B is C₁-C₆ alkyl, preferably C₁-C₂ alkyl, A, C, F, and E are hydrogen, and R₂₂ is C₁-C₆ alkyl, preferably C₁-C₂ alkyl, most preferably methyl.

In certain situations, compounds of Formula I may contain one or more asymmetric carbon atoms, so that the compounds can exist in different stereoisomeric forms. These compounds can 30 be, for example, racemates or optically active forms. In

these situations, the single enantiomers (optically active forms) can be obtained by asymmetric synthesis or by resolution of the racemates. Resolution of the racemates can be accomplished, for example, by conventional methods such as 5 crystallization in the presence of a resolving agent, or chromatography, using, for example a chiral HPLC column.

Representative compounds of the present invention, which are encompassed by Formula I, include, but are not limited to the compounds set forth in Table I and their pharmaceutically 10 acceptable acid and base addition salts. If the compound of the invention is obtained as an acid addition salt, the free base can be obtained by basifying a solution of the acid salt. Conversely, if the product is a free base, an addition salt, particularly a pharmaceutically acceptable addition salt, may 15 be produced by dissolving the free base in a suitable organic solvent and treating the solution with an acid, in accordance with conventional procedures for preparing acid addition salts from base compounds.

Non-toxic pharmaceutical salts include salts of acids 20 such as hydrochloric, phosphoric, hydrobromic, sulfuric, sulfinic, formic, toluenesulfonic, methanesulfonic, nitric, benzoic, citric, tartaric, maleic, hydroiodic, alkanoic such as acetic, HOOC-(CH₂)_n-COOH where n is 0-4, and the like. Non-toxic pharmaceutical base addition salts include salts of 25 bases such as sodium, potassium, calcium, ammonium, and the like. Those skilled in the art will recognize a wide variety of non-toxic pharmaceutically acceptable addition salts.

The present invention also encompasses the acylated prodrugs of the compounds of Formula I. Those skilled in the 30 art will recognize various synthetic methodologies that may be employed to prepare non-toxic pharmaceutically acceptable addition salts and acylated prodrugs of the compounds encompassed by Formula I.

By "lower alkyl" and "C₁-C₆ alkyl" is meant straight or branched chain alkyl groups having the indicated number of carbon atoms when a number is indicated. Examples of such groups are methyl, ethyl, propyl, isopropyl, n-butyl, sec-
5 butyl, tert-butyl, pentyl, 2-pentyl, isopentyl, neopentyl, hexyl, 2-hexyl, 3-hexyl, and 3-methylpentyl.

By "cycloalkyl" and "C₃-C₇ cycloalkyl" is meant cycloalkyl groups having 3-7 atoms such as, for example cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, and cycloheptyl.

10 By "aryl" is meant an aromatic carbocyclic group having a single ring (e. g., phenyl), multiple rings (e. g., biphenyl), or multiple condensed rings in which at least one is aromatic, (e.g., 1,2,3,4-tetrahydronaphthyl, naphthyl, anthryl, or phenanthryl), which is optionally mono-, di-, or
15 trisubstituted with, e.g., halogen, lower alkyl, lower alkoxy, C₁-C₆ haloalkyl, hydroxy, or mono- or di-(C₁-C₆)alkylamino.

In the term "substituted aryl(C₁-C₆)alkyl", it is understood that the aryl group is substituted as provided in the preceding paragraph, i.e., one, two, or three substituents
20 selected from, e.g., halogen, lower alkyl, lower alkoxy, C₁-C₆ haloalkyl, hydroxy, and mono- or di-(C₁-C₆)alkylamino.

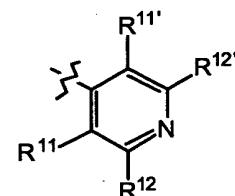
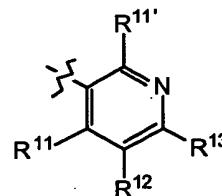
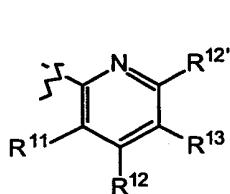
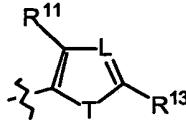
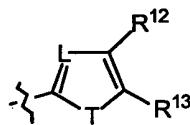
By "lower alkoxy" is meant straight or branched chain alkoxy groups having 1-6 carbon atoms, such as, for example, methoxy, ethoxy, propoxy, isopropoxy, n-butoxy, sec-butoxy, tert-butoxy, pentoxy, 2-pentyl, isopentoxy, neopentoxy, hexoxy, 2-hexaoxy, and 3-methylpentoxy.

By "halogen" is meant fluorine, bromine, chlorine, and iodine.

By "haloalkyl" as used herein is meant C₁-C₆ alkyl groups
30 where one or more hydrogen atoms is replaced by a halogen atom. Representative examples include fluoromethyl, difluoromethyl, cloromethyl, bromomethyl, 1,2-difluoroethyl, trifluoromethyl, perfluoropropyl, 2,2-dichloropropyl, etc. A preferred haloalkyl group is trifluoromethyl.

By "heteroaryl" or "aromatic heterocycle" is meant one or more aromatic ring systems of 5-, 6-, or 7-membered rings containing at least one and up to four hetero atoms selected from nitrogen, oxygen, or sulfur. Such heteroaryl groups 5 include but are not limited to thienyl, furanyl, thiazolyl, imidazolyl, (is)oxazolyl, pyridyl, pyrimidinyl, (iso)quinolinyl, naphthyridinyl, benzimidazolyl, and benzoxazolyl heterocyclic groups. These heteroaryl groups may 10 be unsubstituted or substituted in a substitutable position with one or more groups selected independently from halogen, hydroxy, C₁-C₆ alkyl, C₁-C₆ alkoxy, C₁-C₆ haloalkyl, and mono- or di(C₁-C₆)alkylamino.

Specific examples of heteroaryl groups include:



15 wherein

L is nitrogen or -CR¹¹;

T is -NR¹⁹, oxygen, or sulfur;

R¹¹, R¹², R¹³, R^{11'}, and R^{12'} are as defined above; and

R¹⁹ is hydrogen or C₁-C₆alkyl.

20

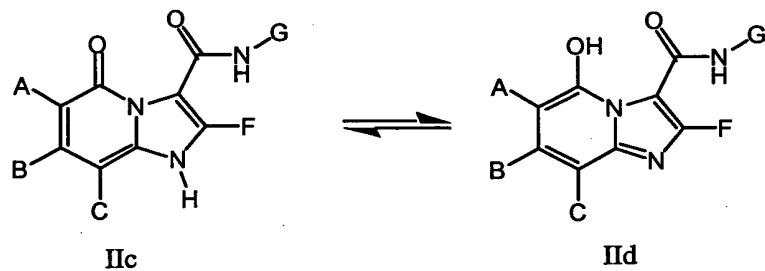
By "heterocycloalkyl" or "heterocyclic ring" is meant saturated or partially unsaturated ring structures having from 3 to about 8 ring atoms, with at least one ring atom selected from nitrogen, oxygen, and sulfur and remaining ring atoms 25 being carbon. Heterocyclic rings may be unsubstituted or substituted with one or more groups such as halogen, hydroxy,

C₁-C₆ haloalkyl, C₁-C₆ alkyl, C₁-C₆ alkoxy, amino, or mono or di(C₁-C₆)alkylamino. Examples of such rings are piperidinyl, piperazinyl, morpholinyl, pyrrolidinyl, imidazolinyl, homopiperazinyl, and homopiperidinyl.

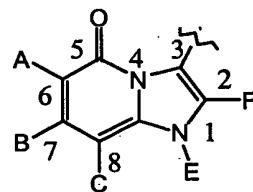
5 In terms such as di(C₁-C₆)alkylamino, di(C₁-C₆)alkylamino(C₁-C₆)alkyl, and di(C₁-C₆)alkylamino(C₁-C₆)alkoxy, it is understood that the selection of each alkyl portion is independent of any other alkyl portion in the group. Thus, for example, di(C₁-C₆)alkylamino includes N-methyl-N-10 propylamino, N-ethyl-N-butylamino, N-methyl-N-ethylamino, etc.

The definition of Formula I as shown in the specification and as used in the claims includes possible isomers, such as tautomers and rotamers. The Formulas IIc and IIId, below, illustrate this point.

15 For Formula I, when E = H:



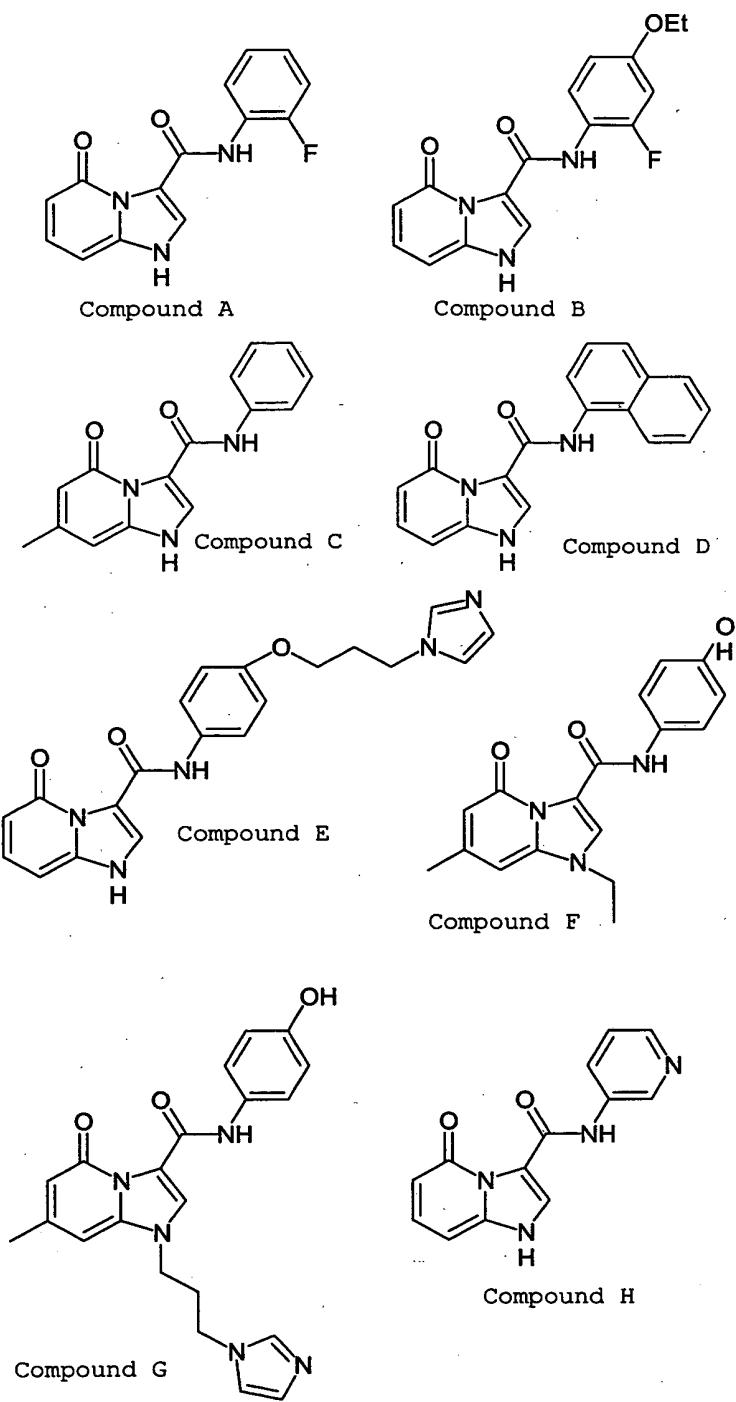
The following numbering system is used to identify positions on the imidazopyridole ring system of the compounds of the invention:



20

Representative compounds of the invention are shown in Table I.

Table I



The compounds of the invention interact with a GABA binding site, the benzodiazepine (BDZ) receptor, as shown in the examples.

This invention provides oxo-imidazopyridine carboxamides 5 that bind, preferably with high affinity, to the benzodiazepine site of GABA_A receptors, including human GABA_A receptors. Preferred compounds are those that show high selectivity for the benzodiazepine site of GABA_A receptors.

The compounds of Formula I and their salts are suitable 10 for the diagnosis and treatment of anxiety, depression, memory impairment, Alzheimer's dementia, Down Syndrome, sleep, cognitive and seizure disorders, and overdose with benzodiazepine drugs and for enhancement of alertness, both in human and non-human animals including companion animals, e.g. 15 domestic pets, especially dogs and cats and livestock animals, e.g., sheep, swine and cattle.

The diseases and/or disorders that can be treated using compounds and compositions according to the invention include:

Depression:

20 depression, atypical depression, bipolar disorder, depressed phase of bipolar disorder.

Anxiety:

25 general anxiety disorder (GAD), agoraphobia, panic disorder +/- agoraphobia, social phobia, specific phobia, Post traumatic stress disorder, obsessive compulsive disorder (OCD), dysthymia, adjustment disorders with disturbance of mood and anxiety, separation anxiety disorder, anticipatory anxiety acute stress disorder, adjustment disorders, cyclothymia

30 Sleep Disorders:

sleep disorders including primary insomnia, circadian rhythm sleep disorder, dyssomnia NOS, parasomnias, including nightmare disorder, sleep terror disorder,

sleep disorders secondary to depression and/or anxiety or other mental disorders, substance induced sleep disorder

Cognition impairment:

5 cognition impairment, Alzheimer's disease, Parkinson's disease, mild cognitive impairment (MCI), age-related cognitive decline (ARCD), stroke, traumatic brain injury, AIDS associate dementia, dementia associated with depression, anxiety or psychosis

10 The invention also provides pharmaceutical compositions comprising compounds of the invention.

The invention further comprises methods of treating patients in need of such treatment with an amount of a compound of the invention sufficient to alter the symptoms of 15 a CNS disorder. Compounds of the inventions that act as agonists at $\alpha_2\beta_3\gamma_2$ and $\alpha_3\beta_3\gamma_2$ receptor subtypes are useful in treating anxiety disorders such as panic disorder, obsessive compulsive disorder and generalized anxiety disorder; as well as stress disorders, including post-traumatic stress and acute 20 stress disorders. Compounds of the inventions that act as agonists at $\alpha_2\beta_3\gamma_2$ and $\alpha_3\beta_3\gamma_2$ receptor subtypes are also useful in treating depressive or bipolar disorders and in treating sleep disorders. Compounds of the invention that act as inverse agonists at an $\alpha_5\beta_3\gamma_2$ receptor subtype or $\alpha_1\beta_2\gamma_2$ and $\alpha_5\beta_3\gamma_2$ 25 receptor subtypes are useful in treating cognitive disorders including those resulting from Down Syndrome, neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease, and stroke related dementia. Compounds of the invention that act as agonists at a $\alpha_1\beta_2\gamma_2$ receptor subtype 30 are useful in treating convulsive disorders such as epilepsy. Compounds that act as antagonists at the benzodiazepine site are useful in reversing the effect of benzodiazepine overdose and in treating drug and alcohol addiction.

In a separate aspect, the invention provides a method of potentiating the actions of CNS active compounds that comprises administering an effective amount of a compound of the invention in combination with another CNS active compound.

5 Such CNS active compounds include, but are not limited to the following: for anxiety, serotonin receptor (e.g. 5-HT_{1A}) agonists and antagonists; for anxiety and depression, neurokinin receptor antagonists or corticotropin releasing factor receptor (CRF₁) antagonists; for sleep disorders,

10 melatonin receptor agonists; and for neurodegenerative disorders, such as Alzheimer's dementia, nicotinic agonists, muscarinic agents, acetylcholinesterase inhibitors and dopamine receptor agonists. In a preferred embodiment, the invention provides a method of potentiating the antidepressant

15 activity of selective serotonin reuptake inhibitors (SSRIs) by administering an effective amount of a GABA agonist compound of the invention in combination with an SSRI.

Combination administration can be carried out in a fashion analogous to that described by disclosed in Da-Rocha, et al., *J. Psychopharmacology* (1997) 11(3) 211-218; Smith, et al., *Am. J. Psychiatry* (1998) 155(10) 1339-45; or Le, et al., *Alcohol and Alcoholism* (1996) 31 Suppl. 127-132. Also see, the discussion of the use of the GABA_A receptor ligand 3-(5-methylisoxazol-3-yl)-6-(1-methyl-1,2,3-triazol-4-yl)

25 methyloxy-1,2,4-triazolo [3,4-a]phthalazine in combination with nicotinic agonists, muscarinic agonists, and acetylcholinesterase inhibitors, in PCT International publications Nos. WO 99/47142, WO 99/47171, and WO 99/47131, respectively. Also see in this regard PCT International

30 publication No. WO 99/37303 for its discussion of the use of a class of GABA_A receptor ligands, 1,2,4-triazolo[4,3-b]pyridazines, in combination with SSRIs.

The present invention also pertains to methods of inhibiting the binding of benzodiazepine compounds, such as

Ro15-1788, to GABA_A receptors which methods involve contacting a compound of the invention with cells expressing GABA_A receptors, wherein the compound is present at a concentration sufficient to inhibit benzodiazepine binding to GABA_A receptors 5 in vitro. This method includes inhibiting the binding of benzodiazepine compounds to GABA_A receptors *in vivo*, e.g., in a patient given an amount of a compound of Formula I that would be sufficient to inhibit the binding of benzodiazepine compounds to GABA_A receptors *in vitro*. In one embodiment, such 10 methods are useful in treating benzodiazepine drug overdose. The amount of a compound that would be sufficient to inhibit the binding of a benzodiazepine compound to the GABA_A receptor may be readily determined via an GABA_A receptor binding assay, such as the assay described in Example 9. The GABA_A receptors 15 used to determine *in vitro* binding may be obtained from a variety of sources, such as, for example, from preparations of rat cortex or from cells expressing cloned human GABA_A receptors.

The present invention also pertains to methods for
20 altering the signal-transducing activity, particularly the
chloride ion conductance of GABA_A receptors, said method
comprising exposing cells expressing such receptors to an
effective amount of a compound of the invention. This method
includes altering the signal-transducing activity of GABA_A
25 receptors *in vivo*, e.g., in a patient given an amount of a
compound of Formula I that would be sufficient to alter the
signal-transducing activity of GABA_A receptors *in vitro*. The
amount of a compound that would be sufficient to alter the
signal-transducing activity of GABA_A receptors may be
30 determined via a GABA_A receptor signal transduction assay, such
as the assay described in Example 10.

The GABA_A receptor ligands provided by this invention and labeled derivatives thereof are also useful as standards and

reagents in determining the ability of a potential pharmaceutical to bind to the GABA_A receptor.

Labeled derivatives the GABA_A receptor ligands provided by this invention are also useful as radiotracers for positron 5 emission tomography (PET) imaging or for single photon emission computerized tomography (SPECT).

Additionally this invention relates to the use of compounds of Formula I as probes for the localization of GABA_A receptors, e.g., in tissue sections.

10 Pharmaceutical Preparations

The compounds of general Formula I may be administered orally, topically, parenterally, by inhalation or spray or rectally in dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and 15 vehicles. The term parenteral as used herein includes percutaneous, subcutaneous, intravascular (e.g., intravenous), intramuscular, or intrathecal injection or infusion techniques and the like. In addition, there is provided a pharmaceutical formulation comprising a compound of general Formula I and a 20 pharmaceutically acceptable carrier. One or more compounds of general Formula II may be present in association with one or more non-toxic pharmaceutically acceptable carriers and/or diluents and/or adjuvants and if desired other active ingredients. The pharmaceutical compositions containing 25 compounds of general Formula II may be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsion, hard or soft capsules, or syrups or elixirs.

Compositions intended for oral use may be prepared 30 according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents selected from the group consisting of sweetening agents, flavoring agents, coloring agents and preservative agents in order to provide pharmaceutically

elegant and palatable preparations. Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients that are suitable for the manufacture of tablets. These excipients may be for example, inert diluents, 5 such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, corn starch, or alginic acid; binding agents, for example starch, gelatin or acacia, and lubricating agents, for example magnesium stearate, stearic acid or talc. 10 The tablets may be uncoated or they may be coated by known techniques. In some cases such coatings may be prepared by known techniques so as to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay 15 material such as glyceryl monostearate or glyceryl distearate may be employed.

Formulations for oral use may also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, 20 calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example peanut oil, liquid paraffin or olive oil.

Aqueous suspensions contain the active materials in admixture with excipients suitable for the manufacture of 25 aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydropropylmethylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents may be a naturally-occurring 30 phosphatide, for example, lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethylenoxyacetanol, or condensation products of

ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example 5 polyethylene sorbitan monooleate. The aqueous suspensions may also contain one or more preservatives, for example ethyl, or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents, and one or more sweetening agents, such as sucrose or saccharin.

10 Oily suspensions may be formulated by suspending the active ingredients in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl 15 alcohol. Sweetening agents and flavoring agents may be added to provide palatable oral preparations. These compositions may be preserved by the addition of an anti-oxidant such as ascorbic acid.

Dispersible powders and granules suitable for preparation 20 of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional 25 excipients, for example sweetening, flavoring and coloring agents, may also be present.

Pharmaceutical compositions of the invention may also be in the form of oil-in-water emulsions. The oily phase may be a vegetable oil or a mineral oil or mixtures of these. 30 Suitable emulsifying agents may be naturally-occurring gums, for example gum acacia or gum tragacanth, naturally-occurring phosphatides, for example soy bean, lecithin, and esters or partial esters derived from fatty acids and hexitol, anhydrides, for example sorbitan monoleate, and condensation

products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monoleate. The emulsions may also contain sweetening and flavoring agents.

Syrups and elixirs may be formulated with sweetening agents, for example glycerol, propylene glycol, sorbitol, glucose or sucrose. Such formulations may also contain a demulcent, a preservative and flavoring and coloring agents. The pharmaceutical compositions may be in the form of a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents that have been mentioned above. The sterile injectable preparation may also be sterile injectable solution or suspension in a non-toxic parentally acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

The compounds of general Formula I may also be administered in the form of suppositories, e.g., for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials are cocoa butter and polyethylene glycols.

Compounds of general Formula I may be administered parenterally in a sterile medium. The drug, depending on the vehicle and concentration used, can either be suspended or dissolved in the vehicle. Advantageously, adjuvants such as

local anesthetics, preservatives and buffering agents can be dissolved in the vehicle.

Dosage levels of the order of from about 0.1 mg to about 140 mg per kilogram of body weight per day are useful in the 5 treatment of the above-indicated conditions (about 0.5 mg to about 7 g per patient per day). The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. Dosage 10 unit forms will generally contain between from about 1 mg to about 500 mg of an active ingredient.

Frequency of dosage may also vary depending on the compound used and the particular disease treated. However, for treatment of most disorders, a dosage regimen of 4 times 15 daily or less is preferred. For the treatment of anxiety, depression, or cognitive impairment a dosage regimen of 1 or 2 times daily is particularly preferred. For the treatment of sleep disorders a single dose that rapidly reaches effective concentrations is desirable.

20 It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, and rate of 25 excretion, drug combination and the severity of the particular disease undergoing therapy.

For administration to non-human animals, the drug or a pharmaceutical composition containing the drug may also be added to animal feed or drinking water. It will be convenient 30 to formulate animal feed and drinking water products with a predetermined dose of the drug so that the animal takes in an appropriate quantity of the drug along with its diet. It will also be convenient to add a premix containing the drug to the

feed or drinking water approximately immediately prior to consumption by the animal.

Preferred compounds of the invention have various pharmacological properties. Such properties include, but are 5 not limited to oral bioavailability, low toxicity, low serum protein binding and desirable *in vitro* and *in vivo* half-lives. Penetration of the blood brain barrier for compounds used to treat CNS disorders is necessary, while low brain levels of compounds used to treat peripheral disorders are often 10 preferred.

Assays may be used to predict these pharmacological properties. Assays used to predict bioavailability include transport across human intestinal cell monolayers, including Caco-2 cell monolayers. Toxicity to cultured hepatocytes may 15 be used to predict compound toxicity. Penetration of the blood brain barrier of a compound in humans may be predicted from the brain levels of the compound in laboratory animals given the compound intravenously.

Serum protein binding may be predicted from albumin 20 binding assays. Such assays are described in a review by Oravcová et al. (Journal of Chromatography B (1996) volume 677, pages 1-27).

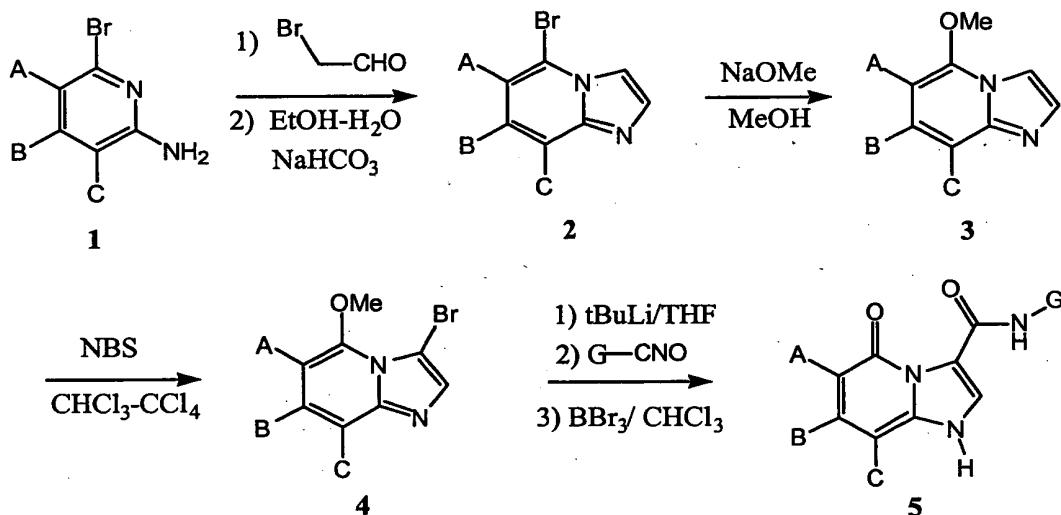
Compound half-life is inversely proportional to the frequency of dosage of a compound. *In vitro* half-lives of 25 compounds may be predicted from assays of microsomal half-life as described by Kuhnz and Gieschen (Drug Metabolism and Disposition, (1998) volume 26, pages 1120-1127).

The present invention also pertains to packaged pharmaceutical compositions for treating disorders responsive 30 to GABA_A receptor modulation, e.g., treatment of anxiety, depression, sleep disorders or cognitive impairment by GABA_A receptor modulation. The packaged pharmaceutical compositions include a container holding a therapeutically effective amount of at least one GABA_A receptor modulator as described herein

and instructions (e.g., labeling) indicating the contained GABA_A receptor ligand is to be used for treating a disorder responsive to GABA_A receptor modulation in the patient.

An illustration of the preparation of compounds of the 5 present invention is given in Scheme 1.

Scheme I:



In Scheme I, the substituents A, B, B and G carry the definitions set forth above for Formula II.

10 The disclosures in this application of all articles and references, including patents, are incorporated herein by reference.

15 The invention is illustrated further by the following examples, which are not to be construed as limiting the invention in scope or spirit to the specific procedures described in them. Those having skill in the art will recognize that the starting materials and reaction conditions may be varied and additional steps employed to produce 20 compounds encompassed by the present invention, as demonstrated by the following examples. Unless otherwise stated, the starting material and reagents employed in these syntheses are of standard commercial grade. In some cases,

protection of certain reactive functionalities may be necessary to achieve some of the above transformations. In general, the need for protecting groups, as well as the conditions necessary to attach and remove such groups, will be 5 apparent to those skilled in the art of organic synthesis.

Representative examples of methods for preparing compounds of the invention are set forth below.

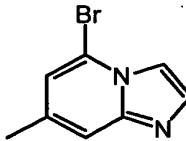
EXAMPLE 1

10 Preparation of starting materials and intermediate

The starting materials and various intermediates may be obtained from commercial sources, prepared from commercially available organic and/or inorganic reagents and compounds, or prepared using well known synthetic methods.

15 Representative examples of methods for preparing intermediates of the invention are set forth below.

Preparation of 5-bromo-7-methylimidazo[1,2-a]pyridine

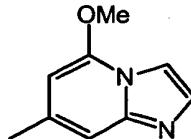


20 A mixture of bromoacetaldehyde diethyl acetal (20 mL), concentrated hydrobromic acid (5 mL) and water (20 mL) is heated at 90°C for 1.5 h. The reaction mixture is poured into 250 mL of water and 50 g sodium bicarbonate is gradually added. 2-Amino-6-bromo-4-methylpyridine (3 g, 0.016 mol) is 25 then added and the mixture is stirred at room temperature for 3 h. Ethyl acetate (100 mL) and water (50 mL) are added. The organic layer is separated and extracted with hydrochloric acid (1N, 3x100 mL). The combined aqueous layer is neutralized with ammonium hydroxide at 0°C and extracted with 30 dichloromethane (3 x 200 mL). The combined organic layer is dried over sodium sulfate. Evaporation of the solvent gave 5-

bromo-7-methylimidazo[1,2-a]pyridine (2.95g, 76%) as a white solid, m.p. 53-54 °C.

EXAMPLE 2

Preparation of 5-methoxy-7-methylimidazo[1,2-a]pyridine

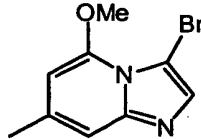


5

A solution of 5-bromo-7-methylimidazo[1,2-a]pyridine (2g, 0.01 mol) and sodium methoxide (25%, 10 mL) in methanol (10 mL) is heated under reflux for 48 h. The solvent is removed and the residue diluted with ethyl acetate (200 mL) and water 10 (150 mL). The organic layer is separated and washed with water and brine, and dried over sodium sulfate. Evaporation of the solvent gave 5-methoxy-7-methylimidazo[1,2-a]pyridine (1g, 62%) as a white semi-solid.

EXAMPLE 3

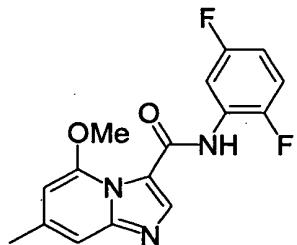
15 Preparation of 3-bromo-5-methoxy-7-methylimidazo[1,2-a]pyridine



To a solution of 5-methoxy-7-methylimidazo[1,2-a]pyridine (900 mg, 5.6 mmol) in carbon tetrachloride (100 mL), is added N-bromosuccimide (990 mg, 5.6 mmol). It is stirred at room 20 temperature for 2h. The reaction solution is then filtered through celite and the filtrate concentrated in vacuo. Ethyl acetate (150 mL) and water are added to the residue. The organic layer is separated and extracted with hydrochloric acid (1N, 3x100 mL). The combined aqueous layer is neutralized 25 with ammonium hydroxide at 0°C and extracted with dichloromethane (3 x 200 mL). The combined organic layer is dried over sodium sulfate. The solvent is removed to give 3-bromo-5-methoxy-7-methylimidazo[1,2-a]pyridine (1.1 g, 78%) as a white solid, m.p. 105-106 °C.

EXAMPLE 4

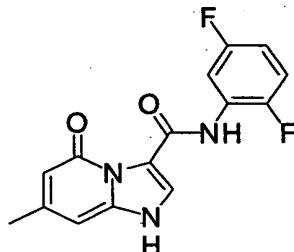
Preparation of N-(2,5-fluorophenyl)5-methoxy-7-methylimidazo[1,2-a]pyridyl-3-carboxamide



5 To a solution of 3-bromo-5-methoxy-imidazo[1,2-a]pyridine (400 mg, 17.5 mmol) in THF (20 mL) is added a solution of t-butyl lithium in pentane (1.5M, 3 mL, 4.5 mmol) at -78°C. The resulting solution is stirred at -78°C under Argon for 5 mins. and is then treated with 2,5-difluorophenyl isocyanate (0.3 mL) at -78°C. The solution is warmed to room temperature and stirred at room temperature for 30 mins. Ethyl acetate (15 mL) and water are added. The organic layer is separated and extracted with hydrochloric acid (1N, 3x15 mL). The combined aqueous layer is neutralized with ammonium hydroxide at 0°C and extracted with dichloromethane (3 x 20 mL). The combined organic layer is dried over sodium sulfate. Evaporation of the solvent gives an oily residue, which is triturated with ether to give N-(2,5-fluorophenyl)5-methoxy-7-methylimidazo[1,2-a]pyridyl-3-carboxamide (302 mg, 57%) as a cream colored solid, m.p. 155-157 °C.

EXAMPLE 5

Preparation of N-(2,5-difluorophenyl)7-methyl-5-oxo-imidazo[1,2-a]pyridyl-3-carboxamide

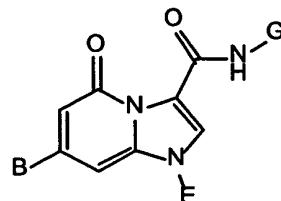


To a solution N-(2,5-difluorophenyl)-5-methoxy-7-methylimidazo[1,2-a]pyridyl-3-carboxamide (110mg, 0.36 mmol) in chloroform (10 mL) is added a solution of boron tribromide in dichloromethane (1.0 M, 2 ml, 2 mmol) dropwise at 0°C. The 5 mixture is then stirred at room temperature for 3h and quenched with methanol. Solvent is then removed in vacuo to dryness. Sodium bicarbonate saturated solution (20 mL) is added to the residue. The solid is filtered, rinsed with water, dichloromethane and dried to give 80 mg of solid 10 product, which is recrystallized from ethanol to give N-(2,5-difluorophenyl) 7-methyl-5-oxo-imidazo[1,2-a]pyridyl-3-carboxamide (Compound 1) as colorless solid. m.p. >285 °C (dec.).

15

EXAMPLE 6

The following compounds of Formula I are prepared essentially according to the procedures described in Examples 1-5:



20

Table II

No.	B	E	G	Name	m.p. (°C)
2	H	H	Phenyl	N-Phenyl 5-oxo-imidazo[1,2-a]pyridine-3-carboxamide	244-246
3	H	H	2-Fluorophenyl	N-(2-Fluorophenyl) 5-oxo-imidazo[1,2-a]pyridine-3-carboxamide	245-247
4	H	H	2-Fluoro-4-chlorophenyl	N-(2-fluoro 4-Chlorophenyl) 5-oxo-imidazo[1,2-a]pyridine-3-carboxamide	215-220
5	H	H	2-Fluoro-3-trifluoromethyl-phenyl	N-(2-Fluoro-3-trifluoromethylphenyl) 5-oxo-imidazo[1,2-a]pyridine-3-carboxamide	274-277 (dec.)

No.	B	E	G	Name	m.p. (°C)
6	H	H	3-Methylphenyl	N-(3-Methylphenyl) 5-oxo-imidazo[1,2-a]pyridine-3-carboxamide	>283 (dec.)
7	H	H	2-Fluoro-5-methylphenyl	N-(2-Fluoro-5-methylphenyl) 5-oxo-imidazo[1,2-a]pyridine-3-carboxamide	280-285 (dec.)
8	H	H	4-Trifluoromethoxy-phenyl	N-(4-Trifluoromethoxyphenyl) 5-oxo-imidazo[1,2-a]pyridine-3-carboxamide	273-275
9	H	H	2-Fluoro-4-ethoxyphenyl	N-(2-Fluoro-4-ethoxyphenyl) 5-oxo-imidazo[1,2-a]pyridine-3-carboxamide	>216 (dec.)
10	H	H	4-(2-Dimethylaminoethoxy)phenyl	N-[4-(2-Dimethylaminoethoxy)phenyl] 5-oxo-imidazo[1,2-a]pyridine-3-carboxamide	198-210
11	H	H	4-(3-Imidazyl-1-propoxy)phenyl	N-[4-(3-Imidazyl-1-propoxy)phenyl] 5-oxo-imidazo[1,2-a]pyridine-3-carboxamide hydrochloride	210-216
12	H	H	2-Naphthyl	N-(2-Naphthyl) 5-oxo-imidazo[1,2-a]pyridine-3-carboxamide	259-265
13	CH ₃	H	Phenyl	N-Phenyl 7-methyl 5-oxo-imidazo[1,2-a]pyridine-3-carboxamide	>275 (dec.)
14	CH ₃	H	4-Fluorophenyl	N-(4-Fluorophenyl) 7-methyl 5-oxo-imidazo[1,2-a]pyridine-3-carboxamide	>274 (dec.)
15	CH ₃	H	4-Hydroxyphenyl	N-(4-Hydroxyphenyl) 7-methyl 5-oxo-imidazo[1,2-a]pyridine-3-carboxamide	>250 (dec.)
16	CH ₃	H	2,4-Difluorophenyl	N-(2,4-Difluorophenyl) 7-methyl 5-oxo-imidazo[1,2-a]pyridine-3-carboxamide	265-268 (dec.)
17	CH ₃	H	2,5-Difluorophenyl	N-(2,5-Difluorophenyl) 7-methyl 5-oxo-imidazo[1,2-a]pyridine-3-carboxamide	284-286 (dec.)
18	CH ₃	H	2-Fluoro-4-hydroxyphenyl	N-(2-Fluoro-4-hydroxyphenyl) 7-methyl 5-oxo-imidazo[1,2-a]pyridine-3-carboxamide	>242 (dec.)

No.	B	E	G	Name	m.p. (°C)
19	CH ₃	CH ₃ CH ₂ -	4-Hydroxyphenyl	N-(4-Hydroxyphenyl) 1-(N-Ethyl) 7-methyl 5-oxo-imidazo[1,2-a]pyridine-3-carboxamide.	
20	CH ₃	3-imidazyl-1-propyl-	4-Hydroxyphenyl	N-(4-Hydroxyphenyl) 1-(3-imidazyl-1-propyl)-7-methyl 5-oxo-imidazo[1,2-a]pyridine-3-carboxamide	

EXAMPLE 7

Preparation of radio-labeled probe compounds of the invention

5 The compounds of the invention are prepared as radio-labeled probes by carrying out their synthesis using precursors comprising at least one atom that is a radioisotope. The radioisotope is preferably selected from of at least one of carbon (preferably ¹⁴C), hydrogen (preferably 10 ³H), sulfur (preferably ³⁵S), or iodine (preferably ¹²⁵I). Such radio-labeled probes are conveniently synthesized by a radioisotope supplier specializing in custom synthesis of radio-labeled probe compounds. Such suppliers include Amersham Corporation, Arlington Heights, IL; Cambridge Isotope 15 Laboratories, Inc. Andover, MA; SRI International, Menlo Park, CA; Wizard Laboratories, West Sacramento, CA; ChemSyn Laboratories, Lexena, KS; American Radiolabeled Chemicals, Inc., St. Louis, MO; and Moravek Biochemicals Inc., Brea, CA.

20 Tritium labeled probe compounds are also conveniently prepared catalytically via platinum-catalyzed exchange in tritiated acetic acid, acid-catalyzed exchange in tritiated trifluoroacetic acid, or heterogeneous-catalyzed exchange with tritium gas. Such preparations are also conveniently carried out as a custom radio-labeling by any of the suppliers listed 25 in the preceding paragraph using the compound of the invention as substrate. In addition, certain precursors may be subjected to tritium-halogen exchange with tritium gas,

tritium gas reduction of unsaturated bonds, or reduction using sodium borotritide, as appropriate.

EXAMPLE 8

5 Receptor autoradiography

Receptor autoradiography (receptor mapping) is carried out in vitro as described by Kuhar in sections 8.1.1 to 8.1.9 of Current Protocols in Pharmacology (1998) John Wiley & Sons, New York, using radio-labeled compounds of the invention 10 prepared as described in the preceding Example.

EXAMPLE 9

Binding Assay

15 The following assay is a standard GABA_A receptor binding assay. The high affinity and high selectivity of compounds of this invention for the benzodiazepine site of the GABA_A receptor is shown using the binding assay described by Thomas and Tallman (J. Bio. Chem. 1981; 156:9838-9842, and J. Neurosci. 1983; 3: 433-440). Essentially the same assay is 20 described below.

25 Rat cortical tissue is dissected and homogenized in 25 volumes (w/v) of Buffer A (0.05 M Tris HCl buffer, pH 7.4 at 4 °C). The tissue homogenate is centrifuged in the cold (4 °C) at 20,000 x g for 20 minutes. The supernatant is decanted, the pellet rehomogenized in the same volume of buffer, and centrifuged again at 20,000 x g. The supernatant of this centrifugation step is decanted and the pellet stored at -20 °C overnight. The pellet is then thawed and resuspended in 25 volumes of Buffer A (original wt/vol), centrifuged at 20,000 x 30 g and the supernatant decanted. This wash step is repeated once. The pellet is finally resuspended in 50 volumes of Buffer A.

Incubations contained 100 µl of tissue homogenate, 100 µl of radioligand, (0.5 nM ³H-Ro15-1788 [³H-Flumazenil], specific

activity 80 Ci/mmol), and test compound or control (see below), and are brought to a total volume of 500 μ l with Buffer A. Incubations are carried for 30 min at 4°C and then rapidly filtered through Whatman GFB filters to separate free and 5 bound ligand. Filters are washed twice with fresh Buffer A and counted in a liquid scintillation counter. Nonspecific binding (control) is determined by displacement of 3 H Ro15-1788 with 10 mM Diazepam (Research Biochemicals International, Natick, MA). Data are collected in triplicate, averaged, and 10 percent inhibition of total specific binding (Total Specific Binding = Total - Nonspecific) is calculated for each compound.

A competition binding curve is obtained with up to 11 points spanning the compound concentration range from 10^{-12} M to 15 10^{-5} M obtained per curve by the method described above for determining percent inhibition. K_i values are calculated according the Cheng-Prussoff equation. Each of the compounds set forth in Examples 5-6 as compounds 1 through 20 is tested in this fashion and each is found to have a K_i of <1 μ M. 20 Preferred compounds of the invention exhibit K_i values of less than 100 nM and more preferred compounds of the invention exhibit K_i values of less than 10 nM.

EXAMPLE 10

25 Electrophysiology

The following assay is used to determine if a compound of the invention act as an agonist, an antagonist, or an inverse agonist at the benzodiazepine site of the GABA_A receptor.

Assays are carried out as described in White and Gurley 30 (NeuroReport 6: 1313-1316, 1995) and White, Gurley, Hartnett, Stirling, and Gregory (Receptors and Channels 3: 1-5, 1995) with modifications. Electrophysiological recordings are carried out using the two electrode voltage-clamp technique at a membrane holding potential of -70 mV. *Xenopus Laevis* oocytes

are enzymatically isolated and injected with non-polyadenylated cRNA mixed in a ratio of 4:1:4 for α , β and γ subunits, respectively. Of the nine combinations of α , β and γ subunits described in the White et al. publications, preferred 5 combinations are $\alpha_1\beta_2\gamma_2$, $\alpha_2\beta_3\gamma_2$, $\alpha_3\beta_3\gamma_2$, and $\alpha_5\beta_3\gamma_2$. Preferably all of the subunit cRNAs in each combination are human clones or all are rat clones. The sequence of each of these cloned subunits is available from GENBANK, e.g., human α_1 , GENBANK accession no. X14766, human α_2 , GENBANK accession no. A28100; 10 human α_3 , GENBANK accession no. A28102; human α_5 , GENBANK accession no. A28104; human β_2 , GENBANK accession no. M82919; human β_3 , GENBANK accession no. Z20136; human γ_2 , GENBANK accession no. X15376; rat α_1 , GENBANK accession no. L08490, rat α_2 , GENBANK accession no. L08491; rat α_3 , GENBANK accession no. 15 L08492; rat α_5 , GENBANK accession no. L08494; rat β_2 , GENBANK accession no. X15467; rat β_3 , GENBANK accession no. X15468; and rat γ_2 , GENBANK accession no. L08497. For each subunit combination, sufficient message for each constituent subunit is injected to provide current amplitudes of >10 nA when 1 μ M 20 GABA is applied.

Compounds are evaluated against a GABA concentration that evokes $<10\%$ of the maximal evokable GABA current (e.g. 1 μ M-9 μ M). Each oocyte is exposed to increasing concentrations of a compound being evaluated (test compound) in order to evaluate 25 a concentration/effect relationship. Test compound efficacy is calculated as a percent-change in current amplitude: $100*((I_c/I)-1)$, where I_c is the GABA evoked current amplitude observed in the presence of test compound and I is the GABA evoked current amplitude observed in the absence of the test 30 compound.

Specificity of a test compound for the benzodiazepine site is determined following completion of a

concentration/effect curve. After washing the oocyte sufficiently to remove previously applied test compound, the oocyte is exposed to GABA + 1 μ M RO15-1788, followed by exposure to GABA + 1 μ M RO15-1788 + test compound. Percent 5 change due to addition of compound is calculated as described above. Any percent change observed in the presence of RO15-1788 is subtracted from the percent changes in current amplitude observed in the absence of 1 μ M RO15-1788. These net 10 values are used for the calculation of average efficacy and EC₅₀ values by standard methods. To evaluate average efficacy and EC₅₀ values, the concentration/effect data are averaged across cells and fit to the logistic equation.

It is to be understood that the foregoing describes 15 preferred embodiments of the present invention and that modifications may be made therein without departing from the spirit of scope of the present invention as set forth in the following claims.